A late blight resistant potato for Europe
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French fries, mashed potatoes, chips, croquettes: these are only a few of the most popular products derived from potato. The potato is a particularly valuable and important crop in Europe. Together we cultivate more than 1.7 million hectares of potato each year. Current cultivation methods come at a high environmental and financial cost. Fungicides are used in huge amounts to protect the potato from late blight, also known as the potato disease.

Late blight is caused by Phytophthora infestans, a fungus-like organism. Most potato varieties have no or only weak defence against Phytophthora. If late blight is not controlled in the field, pre- and post-harvest yields can be lost completely. It is estimated that the yearly economic losses, through crop loss and the cost of control measures, in Europe due to this disease exceed €1 billion.

The most sustainable and environmentally friendly way of cultivating potatoes is to use varieties with resistance to late blight. Thanks to conventional plant breeding (crossing and selecting) several resistant potato varieties have been developed. However currently available resistant varieties compromise on other properties, such as taste, colour, shape, and processability, making them unappealing to consumers and the processing industry. As a result these varieties are hardly cultivated.

Potatoes can also be made disease resistant by genetic modification. The big advantage of this method over conventional breeding is that the elite characteristics of a given potato variety can be conserved. Moreover, combinations of resistance genes can be efficiently transferred in one step leading to a sustainable potato with multiple layers of resistance to late blight.

Several research institutes in Europe aim to introduce late blight resistance to popular potato varieties. The potatoes will have at least three different natural resistance genes obtained from older, wild relatives of the modern potato. This will be achieved using genetic modification. It is estimated that the use of fungicides needed to cultivate these genetically modified potatoes will be at least 80% less than for current conventionally bred varieties that are sensitive to late blight.

**Abstract**

In 2013 Europe produced 53.87 million tonnes of potatoes on 1.74 million hectares. Europe is world leader in potato processing and in development and sale of seed potatoes. Europeans eat up to 80 kg potatoes per person per year. Each year potatoes in Europe are threatened by Phytophthora infestans, a fungus-like organism that causes late blight.

European potato farmers spray their fields on average 15 times per season with several fungicides to control late blight, rising to 20 times per season during humid summers in Belgium, The Netherlands and United Kingdom. In some countries, such as Belgium, potatoes require the greatest use of fungicides of any crop.

One treatment against late blight costs an average of €50 per hectare for a farmer. As well as the economic cost, recurrent chemical spraying causes soil compaction from vehicle tyres and releases excessive CO2 emissions from diesel fuel.

In Europe the estimated economic losses of late blight due to extra costs and production losses exceed €1 billion every year.

Cultivating potato varieties with resistance to late blight is the most environmental friendly and sustainable solution, using 80% less fungicide.

Existing traditionally bred varieties with resistance against Phytophthora infestans do not appeal to consumers or the processing industry due to poor quality traits. As a result they are hardly cultivated.

The identification, isolation and characterisation of new late blight resistance genes have paved the way towards development of resistant potato varieties independent of the breeding method used (crossings or genetic modifications).

Using genetic modification, Wageningen University & Research Centre (The Netherlands) and The Sainsbury Laboratory (United Kingdom) have introduced several late blight resistance genes from wild potato species into the modern potato variety Desiree. These genetically modified potatoes were tested in the field from 2009 onwards in The Netherlands and between 2010 and 2012 in Belgium and the UK.

Based on the positive results of field trials, several research institutes within Europe have started using genetic modification to incorporate late blight resistance in several highly used and well-liked potato varieties. Field trials with these improved varieties are expected from 2017 onwards.
The potato and its disease

Potato is an extremely important crop in Europe, but potato cultivation is threatened every year by late blight. Popular and highly cultivated potato varieties have little to no defence against *Phytophthora infestans*, the fungus-like organism that causes the disease. To protect potato yields, farmers must spray potato fields with fungicides frequently, resulting in significant environmental damage.

From the Andes to Europe

Potatoes originated in South and Middle America.1 There they have been cultivated for more than 7000 years. In 1565 European explorers brought them from the Andes to Europe. However, in the first 200 years upon their arrival, their agricultural use was limited. It was only after several harvest failures of traditional cereals in mid-18th century that potatoes were reinvented as food crop. By the end of that century they were grown on a huge scale across Europe.

Today potatoes are of global interest. Potatoes are grown in almost every country of the world. The only exceptions are equatorial countries lacking a temperate climate even in their mountainous areas; below 10°C and above 30°C tuber growth is restricted. In 2013, 368 million tonnes of potato were produced over 19.5 million hectares.1 China and India together are responsible for more than 36% of the global potato production. In Europe the potato is the most important food crop after wheat. The European potato hectarage is about 1.74 million hectares, producing 53.87 million tonnes of potatoes in 2013.4 Among the highest producers are Germany (11.5 million tonnes), the Netherlands (7 million tonnes), France (6.9 million tonnes), Poland (6.3 million tonnes), the United Kingdom (5.7 million tonnes) and Belgium (4.4 million tonnes).5-7

Potato varieties can be categorised in three different classes based on their characteristics: seed potatoes, consumption potatoes and starch potatoes. Cultivation of seed potatoes is necessary for the propagation of plant material. The Netherlands are the world leader in seed potato production, exporting plant material worldwide. Consumption potatoes are by far the most important in terms of hectarage and production. These are the potatoes we eat, some on a daily basis, as fresh potatoes, chips, and crisps. Finally starch potatoes are cultivated to produce starch for industrial applications: for glues, textile, paper, building materials, etc. One can eat them, but they aren’t tasty. In this report we focus on consumption potatoes only.

Belgium: top potato processor

Over the last ten years the Belgian potato hectarage increased spectacularly from 60,000 to 81,500 hectares (Figure 1).6-8 In 2014 the total Belgian production of consumption potatoes was estimated at 4.58 million tonnes with yields up to 60 tonnes per hectare.8 This record production (because of an increased hectarage and higher yields) is almost 30% higher than the average production, which amounted to 3 million tonnes over the past years.9 Belgian production consists almost exclusively of consumption potatoes. There is no starch potato production and seed potato production is limited. Dozens of different consumption potato varieties are cultivated for specific purposes, such as for mashed potatoes, for crisps, or for home cooking. Each year new varieties are introduced. In Belgium the Bintje potato - the floury variety with yellow flesh that
made Belgium famous for its French fries – remains the most important potato variety in Belgium. (Figure 1) The Bintje accounts for more than half of the Belgian potato hectarage, even though its reputation is declining slightly in favour of varieties such as Fontane and Innovator.

After impressive growth of its potato processing activities Belgium became world-leader. In 1990 only 500,000 tonnes of potato were processed, increasing to almost 3.5 million tonnes in 2013, of which 1.87 million tonnes were reserved for export. Belgium also imports potatoes for processing. Interestingly, in contrast to the general tendency toward industrial globalisation and the increasingly multinational character of the agro-food industry, the Belgian potato processing industry relies on small- and medium-scale, even family, enterprises.

United Kingdom: recovering from 2012

2012 was a terrible year for potato production in the UK. An early drought was followed by low levels of sunlight and high rainfall. Average yields dropped below 40 tonnes per hectare, a 15% reduction for the UK overall (Figure 2). Due to waterlogged ground at harvest, 30% of hectarage was not harvested at the end of October. Late blight infestation was massive and high fungicide use increased variable costs for farmers by 13% compared to 2011. After the devastating year of 2012, UK potato production is back to its standard average production of 5.7 million tonnes per year. In 2014 potatoes were grown on 121,100 hectares and an average yield of 47.4 tonnes per hectare was obtained (Figure 2).

The UK market is more diverse than Belgium in terms of potato varieties. The multipurpose white flesh variety Maris Piper remains the dominant variety, accounting for 16% of the planted area, followed by Markies and Maris Peer. The top five varieties in the UK account for 34% of total planting while Bintje in Belgium has a share of more than 50% on its own. But, as in Belgium, the popular UK varieties are highly susceptible to late blight. UK farmers spend an average of £60 million a year controlling the disease.

More than 90 different varieties of consumption potatoes are registered in The Netherlands. The Bintje variety – still by far the most popular in Belgium – was originally bred in The Netherlands. Today Bintje is hardly grown in The Netherlands due to its high susceptibility to late blight and nematodes. The once so popular variety is now disregarded in favour of Agrina, Victoria, Fontane and Innovator. Agrina is also one of the top varieties in Germany together with Belana, Gala, Fontane and Kuras.

Germany and The Netherlands: seed and starch potatoes

The potato is the most important vegetable in Germany. Consumption volumes (60 kg per person) are double that of tomatoes, the second most popular vegetable. In sharp contrast to Belgium, German potato cultivation area has decreased steadily over the last decade from more than 300,000 hectares in 2000 to 238,000 hectares in 2012. Yet the German potato production went up in 2014 in line with historically high potato production in North West Europe: 11.5 million tonnes produced on 245,000 hectares makes Germany the biggest potato producer in Europe.

Less than half of Germany’s potatoes are planted for consumption (Figure 3). In 2014 seed and starch potatoes took a share of 35% and 24% respectively, at 85,000 and 58,000 hectares. The same situation occurs in The Netherlands. Only 50% of the Dutch planting area is dedicated to consumption potatoes. Of the total Dutch hectarage of 175,000 hectares, starch potatoes account for 30% and seed potatoes for 20%.

*A* While it is botanically a fruit, tomato is considered a vegetable for culinary purposes.
France

‘French fries,’ regardless of the etymology, will always link France to chips. More importantly, France is one of the top potato producers in the European Union. More than 6.5 million tonnes of potatoes are produced yearly on roughly 160,000 hectares. The north of France, Brittany and the central southern region are the most important potato producing regions (Figure 4). France enjoys a diversity of climates, which make growing all types of potatoes (seed, consumption, starch, earlies to maincrop) possible but also the use of a huge amount of different varieties. The most commonly propagated varieties in France are Bintje, Spunta and Charlotte.

France, Belgium, The Netherlands, United Kingdom and Germany make up the top five of highest producing countries worldwide, with a long term average yield of approximately 45 tonnes per hectare. The potato growers of these five countries are united in the North-Western European Potato Growers Foundation.

Global potato cultivation under fire

Potato is known for its susceptibility to many diseases and pests. From nematodes (small parasitic worms) to bacterial diseases (brown rot and ring rot) and late blight, also known as the ‘potato disease.’ Late blight is caused by Phytophthora infestans. This fungus-like organism is the biggest threat to the potato, and tomatoes are also very susceptible to this disease.

Phytophthora develops best under humid conditions between 18 and 25°C; a standard Belgian, British or Dutch summer. Phytophthora infects the leaves, stems and tubers of the potato. Once infected, leaves and stems start to show brown spots mostly surrounded by yellow borders. Typical white fluffy mould can be detected at the underside of the leaves (Figure 5). At optimal conditions for late blight, infected leaves and stems completely die and shivel. Tubers can be infected during growth when spores are washed down from the stems into the soil. Affected tubers show blush stains that later turn rust brown. The infection ends in tuber rot post-harvest that can be directly transmitted to healthy tubers. A small number of tubers infested by Phytophthora can thus lead to large post-harvest losses. According to scientific terminology Phytophthora is a water mould or oomycete, but in this report we refer to it as a fungus-like organism for simplicity.

Late blight is infamous because of Ireland’s great famine of 1845. Two years after Phytophthora arrived in Europe from the United States, the disease caused massive harvest losses in Ireland over consecutive years. The situation was so dramatic that it even profoundly affected Irish history. Approximately one million Irish starved to death and another million migrated to the US.

From the end of the 19th century onwards farmers tried to control late blight by using the famous Bordeaux mixture consisting of copper sulphate and slaked lime. The mixture is a strong pollutant but because of its ‘natural’ origin it is still permitted and used by organic farmers in many European countries.

In the 20th century conventional potato cultivation there was a transition towards more advanced chemical fungicides. However Phytophthora has very flexible and adaptable genetics. This gives it a worrying ability to overcome chemical crop protection agents, or to break through the natural resistance mechanisms in potato. The potential for adaptation is further increased by the enormous number of spores it can produce (more info in the text ‘From spore to spore’, p 11). The rapid propagation of the pathogen worsens matters; more than 20 generations per season can take place in the field.

Figure 4. The North of France, Brittany and the Central Southern region are the most important potato producing regions.

Figure 5. White fluffy mould at the underside of a potato leaf that is infected by Phytophthora infestans.

Statues in Dublin remind of the Great Famine.

A late blight resistant potato for Europe
A late blight resistant potato for Europe

In the 1980s the situation became even worse. Another type of Phytophthora entered Europe and bred with the already established type present. Sexual reproduction further increases the adaptation capacity, making today’s Phytophthora extremely able to overcome many fungicides or resistance mechanisms. Moreover Phytophthora is adapting to the colder climate of central and northern Europe. In Belgium for instance the Potato Research Centre (PCa, Kruishoutem) illustrated that late blight enters the potato hectarage, and the Fontane variety, cultivated both in Belgium and The Netherlands, are both extremely susceptible to late blight. Other highly cultivated varieties in the UK, Germany and The Netherlands - such as Maris Piper, Maris Peer, Agriva and Innovator - are slightly more resistant but still require frequent fungicide treatments to prevent yield losses. Potato farmers in northern Europe typically spray 10 to 15 times per year to control late blight. That number can increase up to 25 times in years with humid summers. Application of agrochemicals increases costs for farmers through chemicals, diesel fuel, machine maintenance and labour, as well as causing soil compaction. In very wet conditions, spraying can be impossible when tractor sprays cannot be used. Phytophthora also causes serious post-harvest losses. In 2008 total costs for late blight control have been estimated in Belgium at €700 per hectare, and €270 million in these three countries alone.

The Bintje variety, accounting for 50 to 60% of the Belgian potato hectarage, and the Fontane variety, cultivated both in Belgium and The Netherlands, is detected earlier in the season (houtem) illustrated that late blight enters the potato hectarage, and the Fontane variety, cultivated both in Belgium and The Netherlands, is detected earlier in the season. As a result the number of advised treatments for Bintje increased to 17 treatments per year (Figure 6), as Phytophthora became more aggressive.

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**ACTIVE INGREDIENT VERSUS FORMULATION**

The biologically active compound in pesticides is called the ‘active ingredient’. Besides the active ingredient, commercially available pesticides (which include herbicides, insecticides, fungicides) contain several other ingredients: solvents to dissolve the active ingredient, dyes, anti-foaming agents, stabilisers to protect the active ingredients but also surfactants to increase the adhesion to and penetration of the plant material. All these products have to undergo risk assessments. In Europe this is done by the European Food Safety Authority (EFSA). Only those products that are safe for farmer, consumer and the environment when used as described by the provider can be placed on the market as a plant protection product. After approval pesticide residues are monitored by the EU member states, which control and report on pesticide residues in the food chain. Nonetheless the fact that EFSA only allows safe products, there is still an increasing public and political demand to further reduce the use of pesticides in agriculture. Plant breeding initiatives and altered cultivation methods can provide alternatives to control plant pests and diseases.

**FROM SPORE TO SPORE**

How is a potato plant infected by Phytophthora? It all starts with spores coming from an infected plant landing on the leaf of a healthy plant. Under conditions favourable for mould growth -moderately warm and humid- the spores germinate and grow thread-like (filamentous) structures, called hyphae. Shortly thereafter an appressorium is formed. This is a specialised pressing structure typical of many fungal and fungus-like plant pathogens that is used to penetrate the leaves of the infected host plant. Once inside the underlying leaf cells a network of filamentous structures grows in and between the plant cells. At several locations in the infected leaf the hyphae enter plant cells and extract nutrients from the plant using a balloon-like structure (haustorium). After a couple of days the hyphae network reaches the underside of the leaf. Hyphae can emerge through the plant’s stomata – openings in the leaf by which the plant exchange oxygen and CO2 with the atmosphere. At the stomata, spore forming structures develop, termed sporangia. These are visible as white, fluffy mould at the underside of infected leaves, where they produce new spores. The spores are then distributed through the wind and rain and can start a new infection cycle. The duration of the whole cycle depends on the weather circumstances. In optimal late blight conditions it takes three to five days. An unnoticed infection in the field can thus infect all surrounding fields in a short period of time.

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**Figure 6. Each year late blight is detected earlier in the field. As a result the number of advised treatments for Bintje increased over the years. Illustration based on observations and advice of the Potato Research Centre (Belgium, Kruishoutem).**
The commercial potato has a problem

The most environmentally friendly way to overcome late blight is to develop and cultivate potato varieties resistant to the potato disease. Susceptibility to Phytophthora differs among potato varieties but only few commercial varieties are really resistant. In contrast to our commercial potatoes, wild potato species from Mid- and South-America do have a strong and natural resistance against late blight (Figure 7).

Mexico seems to be the place of origin for both the potato and the potato disease. Because wild potato species and Phytophthora coexisted for a very long time, wild potatoes had the opportunity to adapt to Phytophthora. Not unexpectedly, the majority of resistance genes to late blight can be found in Mexico (Figure 7). Since the devastating effects of Phytophthora were visible in commercial fields, plant breeders have tried to incorporate resistance genes from wild potatoes using traditional breeding techniques. In some cases only one resistance gene was transferred. Although initially successful, the remarkable adaption potential of Phytophthora rapidly circumvented single resistance mechanisms. In other cases several resistance genes were combined in single potato varieties. Many attempts were done in a ‘blind’ fashion without detailed knowledge of the spectrum of the resistance genes; i.e. the range of Phytophthora isolates that can be recognized. The new breeding products with multiple but narrow-spectrum resistance genes lost their resistance quite easily because of rapidly changing Phytophthora populations. Due to the unsuccessful results and the emergence of fungicides during the middle of the 20th century, the resistance breeding initiatives became less intensive. As a result our most successful varieties Bintje, Maris Piper, Agria have no or only limited resistance against Phytophthora. They are extremely susceptible to late blight.
The wild potato can solve the problem

A wealth of novel resistance genes are being discovered from wild potato species at the sites of origin in for example the Andes. These are resistant to the current Phytophthora isolates (Figure 7). The mechanism in wild potatoes that provides a strong resistance to late blight can be compared with a hypersensitivity reaction.\(^2\) In susceptible plants that have no response to the pest, Phytophthora grows in and between plant cells (for more info see text box ‘From spore to spore’ page 11). During the infection process specific molecules called effectors are produced by the fungus-like organism. The genes which encode these effectors are called avirulence genes. Resistant potatoes are able to recognise these effectors, allowing early detection of Phytophthora (Figure 8).

The long and winding road of potato crossing

Potato breeders have used classical crossing experiments to introduce the natural protection mechanisms of wild potatoes to the commercial varieties. Although effective, this process is time-consuming. Many wild relatives harbouring useful resistance genes are evolutionarily distinct from our potatoes that they cannot be directly crossed anymore.\(^1\) In these situations an intermediate step is needed, more precisely an extra crossing, called bridge crossing. This extra crossing is with a different wild variant, and the crossing product can then be used to cross with a modern potato variety.

When crossing plants half of the genetic material is derived from each one of the parents. This means not only the resistance genes but also a vast amount of other, less wanted traits are transferred; for example low yield or bad taste. It is essential to backcross the crossing product many times again with modern potato varieties until as much as possible desired traits are united in one plant (Figure 9). This gets rid of as many as possible undesirable characteristics of the wild potatoes, to obtain a variety that can satisfy farmer, consumer and processor.

The information underlying this detection system is encrypted in the plant’s resistance genes. Once Phytophthora penetrates a resistant plant cell, the effector is recognised by a resistance protein\(^*\) leading to spontaneous cell death of the surrounding plant cells. This allows the plant to develop a physical border between the infection spot and the healthy plant tissue. The border traps the thread-like structures and stops the march of Phytophthora through the plant.\(^3\) The infestation remains very local and the only visible effect is the presence of small, harmless black spots on the leaves. This mechanism is referred to as a gene-for-gene interaction and can be compared with a key and a lock; a plant is only resistant when the resistance gene of the plant (key) matches with the avirulence gene of the pest (lock). In the absence of the right resistance gene, the pest cannot be detected and the plant becomes sick (Figure 8).

An additional complexity for late blight resistant potato breeders is that the leaves of new potato clones may be resistant to late blight but their tubers might not be, and vice versa; some crossing products have poor foliage resistance but good tuber resistance. It is clear that a commercial variety should ideally have good resistance in both foliage and tuber. After decades of work breeders have obtained a limited number of workable potato varieties that inherited an active resistance against late blight. The varieties Bionica and Toluca are well known examples. They have been obtained after more than forty years of breeding activities.\(^3\) This period was needed to transfer a major resistance gene called BLB-2 (aka Api-blb2 in the scientific literature) from the wild potato Solanum bulbocastanum to a variety with acceptable consumption traits. Recent research revealed that both Bionica and Toluca – besides the BLB-2 resistance gene – possess additional resistance genes obtained from Solanum demissum.\(^3\) However these genes had already lost their effectiveness due to Phytophthora’s adaptation over the years. Having only one effective resistance gene – such as BLB-2 – is a major disadvantage. Because of Phytophthora’s genetic flexibility, a single gene is easily overcome. There are already Phytophthora isolates that succeed

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* The information for the production of a protein is encrypted in a gene. The product of a resistance gene is thus a resistance protein.

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Figure 8. A plant is only resistant when the resistance gene present in the plant is compatible with the avirulence gene from the pest. In all other cases the plant gets infected.

Figure 9. A typical backcrossing scheme to transfer a desired trait (symbolised by the yellow dot, from the purple to the blue parent) but in meantime eliminate as much as possible unwanted characteristics (symbolised by purple dots).
A late blight resistant potato for Europe

Another classically bred variety has a higher potential for late blight resistance. The variety Sarpo Mira has at least five major resistance genes, demonstrating a very high level of late blight resistance for more than ten years. Sarpo Mira was developed by the Hungarian Sárvári family commissioned by the Soviet Union. The Soviet Union wanted a potato variety that could survive the ravages of a harsh climate and disease. As was the case for Bionico and Toluca, Sarpo Mira was a crossing product of commercial and wild South-American and Mexican potatoes. Three resistance genes originate from Solanum demissum and two other genes have an unknown origin.

The development process of Sarpo Mira took more than forty years, but finally the breeders obtained a variety with an exceptionally high resistance toward late blight. However, it remains problematic to predict the extent to which a resistant variety will remain resistant.

A Phytophthora strain that can overcome four of the five resistance genes in Sarpo Mira has already been isolated. It is thus unclear whether Sarpo Mira is resistant because one of the five resistance genes is broad-spectrum and confers the observed field resistance or because Sarpo Mira hasn’t been cultivated widely enough to select for the virulent Phytophthora strain.

Indeed Sarpo Mira is hardly cultivated because resistance to the potato disease is not the only quality necessary for a consumption potato.

Potatoes are mainly grown for their nutritional value, taste and ability to be processed into all kinds of products. On these criteria Sarpo Mira falls short. Disease resistance was held central during the long breeding process, and as a result Sarpo Mira is of poor culinary quality compared to standard consumption varieties. In comparative trials its taste is scored 'insufficient' to 'just sufficient.' Accordingly Sarpo Mira is not popular as fresh potato. Also deep-frying characteristics, important for production of French fries and chips, are substandard. Although the long natural dormancy of Sarpo Mira spuds is interesting because it enables long storage without cooling, the Sarpo Mira foliage has to be cut at a particular point. When left too long, the huge spuds will develop hollow hearts.

New varieties

Despite their increased resistance late blight - and thus reduced need for fungicides - Bionico, Toluca and Sarpo Mira are barely grown. In 2014 their joint hectarage in Flanders was less than 10 hectares, making up 0.0025% of potato hectarage. The most plausible reason is that the characteristics of the currently available varieties are not in line with what the consumers and processing industry expect. However the need for environmentally friendly potato production is pressing. Consequently there is significant demand for new potato varieties with late blight resistance that also have a good yield, taste, and storage and processing characteristics.

Since 2002 the pioneering work of the Sárvári family is developed by the Sárvári Research Trust, a not-for-profit company registered in England and Wales that aims to develop new varieties with high levels of durable blight resistance. The Sárvári family in Hungary still makes the primary selections from their breeding programme which are then further tested and selected by the Sárvári Research Trust at many locations in the UK as well as several other European countries. Additional varieties were developed as Sarpo Uno and Sarpo Shona to meet the demands of the consumers. Yet these new clones perform less compared to the original Sarpo in terms of late blight resistance in field trials.

Field trials executed in 2014 by the Flemish Research and Advisory Centre for Agriculture and Horticulture (Inagro, Beitem, Belgium) revealed a new promising generation of classically bred varieties. Under high disease pressure in the summer of 2014 several varieties - such as Alouette, Carolus, Connect and two other clones yet without a name - showed a very high resistance to late blight. Alouette is a yellow-flesh waxy potato earning high scores as salad potato, while Carolus is a floury potato suited for deep-frying. The Walloon Centre of Agricultural Research (CRA-W, Libramont, Belgium) is breeding potatoes themselves and selected some new varieties that resist Phytophthora. In 2013 the variety Vitabella performed very well.

Sarpo Mira potatoes with the typical red skin.

Sarpo Mira, the survivor from Eastern Europe

A late blight resistant potato for Europe
In the Netherlands, the potato breeding programme Bioimpuls started in 2008. The project is based on a unique collaboration between the Louis Bolk Institute, Wageningen University, commercial breeding companies, potato growers and breeders. It aims to develop new cultivars that are resistant to late blight.

There are without doubt many privately funded research programmes, in addition to the above mentioned initiatives, using classical breeding techniques to obtain a potato variety with a sustainable and strong late blight resistance. These projects are followed with special interest by organic potato farmers and industry. Organic potato farming accounts for 500 hectares in Belgium and 2,000 hectares in the Netherlands. Currently, certified organic farming does not allow the use of genetically modified (GM) crops. In this sector, classically bred varieties are the only option for late blight resistance. However, this does not imply that investing in plant biotechnology and developing GM crops would not be beneficial for organic farming. Molecular biology and DNA technology are needed to identify, isolate and characterise new resistance genes and this knowledge is not used exclusively to introduce a GM potato in the field. Also classical breeding is taking advantage of the molecular insights obtained thanks to GM research.

Without molecular knowledge of resistance genes, late blight resistance could only be detected after intentional infection of plants with Phytophthora. Plants that resist infection are most probably those that inherited one or several resistance genes. Thanks to plant biotechnology research it is now known which specific genes are key to resistance to specific Phytophthora isolates and which genes distinguish races of the pathogen. Using DNA analysis techniques, these genes can be tracked directly in crossing products thereby accelerating the traditional breeding process significantly. Consequently, this marriage between classical breeding and plant biotechnology is becoming more the rule than the exception.

A late blight resistant potato for Europe

MANAGEMENT AND GOOD CULTIVATION PRACTICES

Cultivating Phytophthora resistant potato varieties can significantly reduce economic and environmental damage. But alternative cultivation practices and waste management can also reduce this damage. Removing volunteer potatoes in the field and destroying potatoes in compost and scrap heaps are important measures to lower the disease pressure. In some countries such as The Netherlands, it is even dictated by the government because both infected spuds and volunteers are perfect material to inoculate freshly emerged potato plants with Phytophthora early in the season. Additionally, extending crop rotation (potato cultivation only once in three or four years on the same plot) can help fight late blight because some types of spores can still start a new infection even after being dormant for several years. A third way to escape from the potato disease is the use of early potato varieties. Early cultivars can sometimes be harvested before late blight strikes massively in mid-May or the beginning of June.

* A volunteer is a plant that grows on its own, rather than being deliberately planted by a farmer. Volunteers often grow from seeds or tubers that were unintentionally left on the field and were able to germinate or sprout the year after.

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3 Genetic modification as efficient crossing alternative

Late blight resistance genes from wild potatoes can be introduced in our commercial potatoes by two different ways: using classical breeding or through genetic modification. In both cases the same resistance genes are built into the DNA of the modern potato. Only the way in which it is done differs.

Based on the cultivation of classically bred potato varieties Bionica, Toluca and Sarpo Mira it becomes clear that the use of late blight resistant cultivars is the ideal way to face the potato disease and to reduce fungicide sprayings. However it is essential to note that the use of late blight resistant potatoes will not eliminate all fungicide use. Potatoes are attacked by many different fungi and fungus-like organisms. Without doubt Phytophthora is the most economically important one. Cultivating Phytophthora resistant potatoes will drastically reduce the overall fungicide sprayings, but without additional breeding initiatives to introduce resistance to other pests (e.g. the fungus Alternaria), fungicides will remain necessary, even in the late-blight resistant potato cultivation.

From the available late blight resistant varieties, two of them – Bionica and Toluca – have only a limited protection against Phytophthora. A sole resistance gene – even when broad-spectrum* - is unlikely to provide a durable defence. Phytophthora has a tendency to break through single defence mechanisms quickly. Consequently those potato varieties with a single resistance gene rapidly lose their major advantage. In contrast, combining several functional resistance genes - as in Sarpo Mira, for example - can be more effective and may offer durable protection, as it is more difficult for Phytophthora to circumvent multiple defence mechanisms at once.

To achieve sustainable potato production, three or more distinct resistance genes are needed in one potato variety. Sarpo Mira promisingly possesses five different resistance genes although their current effectiveness is unclear (see earlier page 13). But Sarpo Mira does not have accepta-

*broad-spectrum is used for a resistance gene that delivers protection against a large number of different Phytophthora isolates.

A tasty potato with durable resistance

During the past years many genes that deliver late blight resistance in wild potato species have been mapped and characterised. These genes can be introduced directly in the commercial varieties using gene technology. The result is a resistant potato that still retains all specific variety characteristics. In contrast to classical breeding methods, genetic modification does not mix other traits of the wild potato with those of the commercial one. Only one or a few specific attributes are introduced. GM Bintje stays Bintje and GM Maris Piper stays Maris Piper, with the exception that the GM variants will be able to resist late blight.
A public initiative from the United Kingdom

One of the priorities of The Sainsbury Laboratory in the United Kingdom is to study the interaction between Phytophthora infestans (causing late blight) and potato plants. This knowledge can be used to develop late blight resistant potatoes. Over the years The Sainsbury Laboratory has identified and isolated several different late blight resistance genes from wild potatoes that originate from Mexico and South-America. These genes have also been characterised and introduced using genetic modification into the variety Desiree. Desiree is a relatively popular all-rounder in the UK and in The Netherlands but highly susceptible to late blight. It is also a much-used variety in genetic research because Desiree can be genetically modified relatively easily. A series of GM Desiree potato plants with two different single resistance genes were tested in the fields of Norfolk at the John Innes Centre in 2010, 2011 and 2012.

In 2012 the conditions for late blight were ideal and Phytophthora struck hard. The non-modified Desiree plants were 100% infected and perished completely. The GM Desiree however remained highly resistant to the end of the experiment and yielded more than double the amount of potatoes compared to the susceptible potatoes. The results of such field trials are essential for investigating which resistance gene can provide strong protection against late blight. However, given the problems outlined above with using only one resistance gene, it is not wise to commercialise these resistant Desiree potatoes. Phytophthora could easily overcome this single resistance mode-of-action. The GM potato would lose its major advantage and the resistance gene would no longer be useful in future breeding strategies. Since the number of resistance genes is not endless, they should be handled with care. The most efficient way to develop a potato with durable resistance is to stack or assemble several resistance genes with different modes of action. So instead of using one resistance gene as in Bionica and Toluca, or two genes as in Fortuna (see text box ‘The paralysing effect of the European approval procedure and the anti-GM attitude’ page 19), The Sainsbury Laboratory aims to combine multiple resistance genes to make Desiree and Maris Piper varieties that can completely withstand attacks from late blight. Such a mission can only be accomplished when using the newest breeding technologies; more precisely, genetic modification.

THE PARALYSING EFFECT OF THE EUROPEAN APPROVAL PROCEDURE AND THE ANTI-GM ATTITUDE

The company BASF Plant Science developed Fortuna, a Phytophthora resistant genetically modified potato. Two major resistance genes from Solanum bulbocastanum – BLB-1 and BLB-2 – were incorporated in the DNA of Fontane, a high yielding multi-purpose potato with floury cooking qualities, suitable for fries and chips. In both Flanders (Belgium) and The Netherlands it is the second most important variety. The GM Fontane is identical to Fontane, apart from a natural protection mechanism against late blight transferred to Fontane with the help of genetic modification. The Fontane potato has been tested in the field since 2006 on twenty different locations in Germany, The Netherlands, Belgium, The United Kingdom, Czech Republic and Sweden. In the five years of intensive testing during which the potatoes were exposed to high concentrations of different Phytophthora isolates, the plants remained healthy. On October 31st 2011 BASF submitted a request with the European Commission to grow the Fontane potatoes commercially and use them in food and feed. However because of the European anti-GM attitude, on both political and social level, BASF decided to stop its investments in Phytophthora resistant potatoes for the European market and in January 2013 the company retracted their dossier from the ongoing approval procedure. European farmers are waiting for a popular variety with late blight resistance, potato production would be less dependent upon fungicide treatments, with resulting environmental benefits, but the genetically modified potato of BASF is unlikely ever to reach European farmers.
The cisgenic approach from The Netherlands

In 2006 Wageningen University & Research Centre (The Netherlands) started a 10-year research project commissioned by the Ministry of Agriculture, Nature and Food Quality. The goal is to develop a prototype of a late blight resistant potato variety. The DuRPh project uses the Desiree variety and the technique of genetic modification. But Wageningen decided to use cisgenesis. Cisgenesis means introducing genetic information from plants that are cross-compatible with our modern potato. Because of this restriction the researchers are not allowed to use selection genes (see Text box ‘Selection genes’). These are genes - mostly derived from bacteria - introduced into the plant DNA together with the genes of agricultural interest; in this case, resistance genes against late blight. Selection genes facilitate the selection of modified plantlets following the genetic modification protocol (see Text box ‘Cisgenesis versus transgenesis’).

Researchers at Wageningen UR have been conducting field trials since 2009, using different cisgenic varieties (Desiree, Première, Aveka and Atlantic) which carry different single or multiple resistance genes originating from various wild species. Several years of work have shown that the varieties carrying only one late blight resistance gene may become infected during the growing season while those with at least two or more resistance genes were resistant for the entire season. The Wageningen UR strategy for deployment of late blight resistance genes also explicitly includes management of the resistance genes based on monitoring of the local Phytophthora population and its adaptation to available late blight resistance genes. Additionally a “no spraying unless needed” strategy is followed to open up the possibility to apply fungicides when cultivar resistance depends on an insufficient number of effective resistance genes. In the latter case, controlled applications of fungicides at significantly reduced dose rates were shown to be more than effective and can be used to ensure a long use of the deployed resistance genes.

A BintjePLUS for Belgium

The single and multiple resistant GM Desiree potatoes developed by Wageningen UR were also tested in Flanders fields. A project-driven consortium of three institutes (Ghent University, VIB and ILVO) wanted to test the possibility of using multiple resistant potatoes to combat late blight. A field trial was organised in 2011 and 2012. The summer of 2011 was dry and warm, conditions which are not favourable for late blight. The potato disease had to be artificially introduced in the test plot. In contrast 2012 had a humid summer and Phytophthora isolates circulating in Flanders spontaneously blew in. In both years the susceptible varieties (Bintje, Agria, Fontane, non-GM Desiree) were heavily infected. The single resistant potatoes -both genetically modified as non-GM (Bionica-) performed well but at the end of the season they became infected. Especially in 2012 Bionica and Toloca showed a significant infestation, suggesting that some Phytophthora races already evolved the ability to overcome BLB-2 mediated resistance. Only the multiple late blight resistant potatoes (Sargo Mira, the wild Solanum bulbocastanum and triple resistant GM Desiree plants) remained 100% healthy.

Motivated by the positive results of the Flemish potato field trial, the Belgian researchers initiated the BintjePLUS-project. Started in 2014 this project aims to develop a multiple late blight resistant potato of the Bintje variety. At least three and preferably four distinct resistance mechanisms will be combined in Bintje using genetic modification. The first field trials are expected in 2017.

DESTRUCTION BY ACTIVISTS

In March 2011, one week after the Belgian field trial was authorised the researchers were confronted with the Belgian Field Liberation Movement (FLM). The organisation announced that they would destroy the field trials on May 29th 2011. The scientists invited FLM for meetings and discussion but the anti-GM movement did not accept the invitations. Additionally, FLM representatives were invited to plant classically bred potatoes next to the field trial, on the condition that the activists would not destroy the GM field trials. But also this invitation was refused. Provoked by more and more specific threats the Belgian researchers were forced to take serious measures to protect the field. After consultations with the police a double fence surrounding the plot was installed in addition to camera monitoring and permanent surveillance. These additional safety measures doubled the field trial budget. However these measures which might be interpreted at first sight as overkill turned out to be insufficient. Even a police force of 86 men could not prevent a number of activists from entering the field by force. The activists destroyed 15% of the field trial. The Belgian researchers invested much time and effort restoring the field. Thanks to financial support from the Flemish government, the field trial was largely saved. In December 2014 eleven activists were convicted by the court in Ghent. The judge decided that anti-GM activists are entitled to express their concerns, but destroying an authorised field trial cannot be justified for any reason.
A late blight resistant potato for Europe

cisgenic potatoes should follow the European GMO legislation. Nor do we suggest that in this document we report on genetically modified potatoes. Sometimes we use the term cisgenic potato but we do not categorise these potatoes as GMOs according to the legal European definition. Currently this issue is being discussed in Europe.

WHY A GENETICALLY MODIFIED PLANT IS NOT ALWAYS A GMO

In 1983 Belgian and American scientists described a new method to introduce genetic information into plants without the need for sexual reproduction. This method was named genetic modification and the obtained genetically modified plants. In the nineties governments introduced specific legislation to control the use of the new technology mainly because there was little experience with it at that time, and because it significantly opened up breeding possibilities. To capture these plants in legislation a standard definition was needed. For a genetically modified organism or GMO the definition is: “an organism in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating and/or natural recombination”. Because of this definition the term ‘GMO’ gets a legislative character and therefore only applies in specific situations.

Genetic modification allows transfer of genetic information across the species barriers (transgenesis) but it can equally be used to exchange genes within the same species (cisgenesis). In the latter case the result resembles very much that of a traditional crossing product. Therefore one can question whether cisgenic plants should fall under the legal GMO definition. Currently this issue is being discussed in Europe.

In this document we report on genetically modified potatoes. Sometimes we use the term cisgenic potato but we do not categorise these potatoes as GMOs according to the legal European definition. Nor do we suggest that cisgenic potatoes should follow the European GMO legislation.

SELECTION GENES

Genetic resistance against the potato disease can be introduced through crossings or genetic modification. In both cases this doesn’t occur with 100% efficiency. Because late blight resistant potatoes look identical to susceptible potatoes, it is necessary to have methods to identify which plants received the new genetic information. In traditional breeding programmes all plants (also the susceptible ones) are kept and only enough that are infected with Phytophthora. The plants which show greater resistance are then – most probably – the plants which inherited one or more resistance genes. An alternative and faster method is to check for the presence or absence of specific resistance genes at a very early stage of development. This is done with DNA analysis techniques. The term ‘marker assisted selection’ is often used to refer to this method. The only disadvantage of the traditional method is its labour-intensive, as hundreds or thousands of plantlets must be tested to find the resistant ones.

For the selection of genetically modified plants a much more efficient method can be used. Instead of crossings, one can simply choose which DNA fragment will be introduced into plants. Besides DNA fragments that deliver resistance against late blight, it is possible to incorporate additional DNA that makes the selection of genetically modified plants much easier. These genes – often referred to as selection genes – code for example for resistance against a certain herbicide or antibiotic. As a result, after genetic modification one can grow the plants in the presence of the herbicide or antibiotic. Those that survive have incorporated the new DNA in their genome. This selection is only used within the lab; the antibiotic or herbicide will not be used in the field.

In traditional breeding programmes all plants (also the susceptible ones) are kept and when old enough they are tested for resistance against late blight. Because of this definition the genes should also be accompanied by their original expression signals; the switches that decide when genes are on or off and to which extent. Cisgenic plants are not allowed to contain foreign selection genes (see Text box ‘Selection genes’). Selection genes can still be used in the process of genetic modification but they should be removed from the genome later on. This introduces an extra technical step, which in practice means that selection genes are hardly used when making cisgenic plants. As a result the cisgenic selection process is much less efficient when compared to transgenesis.

Because the crossing of natural species boundaries is avoided, the general public is intuitively more enthusiastic about cisgenesis. Yet regarding the environment and food safety issues, there is no difference between cisgenesis and transgenesis. In the risk assessment discussion the trait should be central, meaning for which characteristic(s) the introduced gene(s) encodes. The species from which the DNA is used (and whether it is crossable or not) is of no importance whatsoever in this respect. The only important reason to make the distinction between cis- and transgenesis is the regulatory issues (see Text boxes ‘The road to commercialisation of the genetically modified potato’).

CISGENESIS AND TRANSGENESIS

All commercially grown genetically modified plants today are transgenic plants. ‘Trans’ refers to material which has originated from another group, for these plants a DNA fragment has been introduced that is not present within the same species. For example, the bacterial St gene in insect resistant cotton (see VIB Fact Series ‘Bt cotton in India’), the bacterial EPSPS gene in herbicide tolerant soy (see VIB Fact Series ‘Yenibreider tolerant soy in Argentina’) or the viral coat protein gene in virus resistant papaya (see VIB Fact Series ‘Virus resistant papaya in Hawaii’). But also introducing a maize gene to rice makes the rice transgenic. Only when DNA is transferred within crossable species, called breeder’s gene pool – for example from rice to rice or from wild potato species to our commercial potato – then the term ‘cisgenic’ can be used. ’Cis’ refers to within the same group. To meet this definition the genes should also be accompanied by their original expression signals, the switches that decide when genes are on or off and to which extent. Cisgenic plants are not allowed to contain foreign selection genes (see Text box ‘Selection genes’). Selection genes can still be used in the process of genetic modification but then they should be removed from the genome later on. This introduces an extra technical step, which in practice means that selection genes are hardly used when making cisgenic plants. As a result the cisgenic selection process is much less efficient when compared to transgenesis.

A late blight resistant potato for Europe
Genetic modification can achieve what classical potato breeding is failing to do: introducing resistance to Phytophthora without changing the characteristics which underlie the success of a variety. Furthermore genetic modification is significantly faster than traditional crossings. Nevertheless the development and testing in the field of genetically modified plants takes several years.

**Phase 1:**
**Identification of resistance genes**

In order to make potato varieties resistant to Phytophthora, late blight resistance genes should first be identified. Wild potato species can be used because they regularly possess several resistance genes. However, the presence of multiple resistance genes makes it difficult to evaluate their individual contributions to the overall resistance level. Therefore in a first step researchers try to separate one of the resistance genes from wild potatoes by crossing them with a modern susceptible potato. Once this is done, the characterisation can start. One of the most important things is knowing to which specific Phytophthora isolates the genes provide resistance. This can be done using leaf assays (see Text box ‘Studying the action of resistance genes through leaf assays’). If the characteristics of the resistance genes are promising (i.e. high and broad spectrum resistance) then several DNA analysis techniques are used to localise the gene and ultimately to isolate it. During the last decade several European research institutes have identified resistance genes from wild potatoes and have already tested them in the field.44,45

**Phase 2:**
**Transferring resistance genes to well-appreciated varieties**

The UK labs want to develop a late blight resistant Maris Piper potato while the Belgian consortium aims to provide a Bintje potato with a durable Phytophthora resistance. For the Dutch initiative it has yet to be seen which potato variety or varieties will be selected to translate the gathered knowledge from the GM Desiree field trials. Phase 2 of these projects consists of building several resistance genes - individual and in combination - into the DNA of the chosen varieties. To achieve this, the scientists make use of the natural DNA-transfer mechanism of the bacterium Agrobacterium tumefaciens. Thus, the resistance genes first have to be transmitted to Agrobacterium.

The Agrobacterium then has to be brought into contact with the potato tissue. Small pieces of potato plants are incubated in sterile culture dishes. They are able to grow in vitro on a matrix which contains all substances needed for plant growth. Next the plant tissue is infected with the modified Agrobacteria. The bacteria subsequently introduce the resistance gene(s) to a number of plant cells. These plant cells can then incorporate the resistance genes into their chromosomes. The last step of phase 2, i.e. the regeneration of a potato plant, is possible thanks to a unique ability of plants to grow an entire organism starting from one cell only. Adding the necessary plant hormones to the matrix stimulates plant tissue to regenerate new shoots. Generally plant shoots will spontaneously develop roots.

**Phase 3:**
**Functional testing of resistance genes**

In phase 3 the functionality of the resistance genes is analysed in the chosen varieties (e.g. Bintje or Maris Piper) obtained in phase 2. Resistance genes that work in, for example, Desiree are not necessarily active in other potato varieties. The genetic background of the variety can have a significant impact.
Phytophthora can appear in distinct isolates or races comparable to plant varieties and animal breeds. Resistance genes towards late blight can be broad range or narrow spectrum. Broad range means that the protection mechanism is active against multiple Phytophthora isolates simultaneously, while a narrow spectrum mechanism will only recognise one or a limited number of Phytophthora isolates. This can be determined using leaf assays as in phase 1. To come up with a final selection of resistance genes the effectiveness of the single resistance genes and that of their combinations have to be assessed, in order to develop a potato with durable resistance. Resistance genes expressed together in one plant can either broaden or narrow down the resistance level compared to the single resistance level.

STUDYING THE ACTION OF RESISTANCE GENES THROUGH LEAF ASSAYS
To analyse whether the resistance mechanisms of the genetically modified potatoes recognise the effectors (products of avirulence genes) of distinct Phytophthora isolates an Agrobacterium-mediated leaf assay is often used. Agrobacterium was already mentioned in the report as the bacterium that naturally transfers DNA to plant cells during the process of genetic modification (page 24). However Agrobacterium can also be exploited to temporarily produce certain proteins in plant cells. To check the functionality of late blight resistance genes single avirulence genes of Phytophthora isolates will be transferred to distinct Agrobacterium strains. For every avirulence gene, another Agrobacterium strain. The next step is to infect the leaves of the single or multiple resistant genetically modified potatoes with one or more of the Agrobacterium strains. This is done by agro-infiltration, a technique to inject Agrobacterium in the leaf in such a way that the bacteria can spread between the plant cells. Once in the leaf Agrobacterium will infect certain cells which then will transiently produce the corresponding avirulence proteins or effectors. If the available resistance genes in the genetically modified plant are tailored to the avirulence genes a hypersensitivity reaction will be visible on the leaf. This response is visible to the bare eye as a zone of dead plant cells. This relatively easy though specific analysis method allows determining the functionality and the range of individual and combined resistance genes.

A late blight resistant potato for Europe

PHASE 4: developing and testing resistant varieties
In phase 4 the genetically modified potatoes with optimal combination of late blight resistance genes will be selected and extensively tested in the greenhouse and in the field. If a transgenic approach is chosen then researchers will select plants from the population obtained in phase 3. For cisgenesis however, plants cannot hold selection genes (see Text box ‘Cisgenesis versus transgenesis’ page 23). In this case new genetically modified potato plants will be developed using the knowledge obtained in phase 3 but without using selection genes.

After identifying several potato lines with the highest and broadest level of resistance to late blight, it will be of utmost importance to study the variety characteristics of the genetically modified potatoes. Therefore the morphology of the leaves and stems, the size and shape of the tubers, and the overall growth and development will be evaluated. These characteristics of the GM Bintje or GM Maris Piper should not diverge too far from what can be expected of a conventional Bintje or Maris Piper variety. The genetically modified potatoes should resemble as much as possible the variety which was used as starting material, except for the late blight resistance. However there is a fair chance that during the process of genetic modification, plant regeneration and in vitro propagation the variety characteristics will be influenced. When Agrobacterium builds a DNA fragment into a plant’s DNA
the function of certain traits can be affected. This probability is rather low since potato is a tetraploid, meaning that there are four copies of every gene. If one copy would no longer function due to the insertion, there are still three other copies that can take over the job. But also because of the growth phase in vitro, small potato plantlets may display noticeable changes that originate from small DNA modifications. This commonly observed phenomenon is called somaclonal variation. This phenomenon is not linked to the technique of genetic modification, since it also occurs during in vitro propagation of non-GM potatoes. Nonetheless, the obtained genetically modified potatoes should undergo a very stringent selection to exclude the possibility that during all the development steps some important variety specifics have been altered. During this selection process in the greenhouse, plants will be evaluated on all important variety characteristics and on their resistance level towards late blight. Only those plants that score well on both levels qualify to be tested in the field.

Finally field trials will be initiated to study the performance of a selected number of genetically modified potatoes in the field. In contrast to evaluation in greenhouses - where only the leaves are exposed to the avirulence genes of Phytophthora - the entire plant will then be exposed to the naturally present Phytophthora population. Not only foliage resistance but also tuber resistance will then be analysed. Additionally the yield and the effect of other factors (wind, rain, drought, infections with fungi, bacteria, viruses or insects) will be addressed.

The genetically modified Bintjeas and Maris Pipers should not only perform well in the field, but also regarding culinary and processing properties. The different potato lines from the field trial will thus be tested on their taste and processing characteristics, for example frying quality. Ultimately, it will be the overall score regarding late blight resistance, performance in the field, yield, taste and processing characteristics of the individual potato lines that will determine which GM potato line will be selected for commercialisation.

The genetically modified potatoes will have to undergo an extended testing phase in the field, since it also occurs during field trials, since it also occurs during field trials. This phenomenon is called somaclonal variation. This phenomenon is not linked to the technique of genetic modification, since it also occurs during the growth phase in vitro. Small potato plantlets may display noticeable changes that originate from small DNA modifications. This commonly observed phenomenon is called somaclonal variation. This phenomenon is not linked to the technique of genetic modification, since it also occurs during in vitro propagation of non-GM potatoes. Nonetheless, the obtained genetically modified potatoes should undergo a very stringent selection to exclude the possibility that during all the development steps some important variety specifics have been altered. During this selection process in the greenhouse, plants will be evaluated on all important variety characteristics and on their resistance level towards late blight. Only those plants that score well on both levels qualify to be tested in the field.

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THE WAY TOWARDS COMMERCIALISATION OF THE GENETICALLY MODIFIED POTATO

Breeding a new variety is one task. Then the variety needs to be prepared for commercialisation, which can be particularly challenging for genetically modified crops. For a new variety obtained using traditional breeding techniques, the latter procedure is rather easy and straightforward. Field trials determine the agricultural value and the quality aspects of the variety. These analyses are necessary to allocate the new variety to national catalogues. An additional comparative analysis with the varieties already available is required to obtain plant breeders’ rights. After this the testing phase can be closed. Some additional tests are needed concerning potato in certain countries to ensure that the concentration of specific toxic compounds naturally present (e.g. glycoalkaloids) does not exceed a strictly defined threshold. The new variety can then be made available to farmers following propagation of the planting material. However, for crops covered by the European GM legislation there are many more procedures to go through. Transgenic plants - those which received DNA from another species, e.g. a gene from maize into rice - are subject to GM-legislations. As described above, by definition, GM crops are plants that are the result of a genetic modification which it is not possible to achieve with classical crossings or spontaneous modification of the DNA. In that case the producer should submit a voluminous dossier to the European Commission reporting on the results of a long list of predetermined experiments. This includes molecular characterisation of the transgenic plant, detailed comparative studies between the plant and its non-GM equivalent, and toxicity and allergenicity studies. Moreover, the final decision of whether a transgenic plant can be cultivated and/or used in the EU doesn’t rely on scientific arguments alone. The ultimate approval is given by political representatives of European member states.

Phytophthora resistant transgenic potatoes have a long approval procedure ahead. But for potatoes developed using cisgenesis the procedure may be different. Natural resistance genes obtained from wild potato relatives can be introduced by crossings with modern potatoes. The traditionally bred varieties Bionica, Toluca and Sarpo Mira are the living examples. In case of cisgenesis genetic modification is only used to provide an existing variety, such as Bintje, with a natural protection mechanism from wild potatoes, rather than to introduce genes from other species. Since cisgenesis does not use bacterial selection genes the Phytophthora resistant potato obtained this way is in theory indistinguishable from a traditionally crossed potato. It is thus fair to question whether these genetically modified cisgenic potatoes should still follow the elaborate risk assessment created for transgenic plants. If they do have to fulfil all criteria then a time-consuming and expensive approval procedure awaits. If we want to ensure that genetically modified crops are put on the market not exclusively by multinational companies, but also by small farmers and seed producers, then we should dare to consider simplifying the current procedure, especially in cases like the cisgenic late-blight resistant potato.

* In The Netherland, US and Sweden this is an obligation, but for example in Belgium this is not the case.

** It is impossible to provide late blight resistance to an existing variety as Bintje using traditional breeding techniques or crossbreeding without changing the variety characteristics. This is because Bintje (as well as other potato varieties) is the result of a very heterogeneous combination of genes. Crossings mix both father- and mother-DNA to a new combination, the original genetic combination can never be obtained anymore. Even back-crossings will not help in case of a heterogeneous genotype. Moreover many varieties like Bintje are almost or completely sterile which makes crossings very difficult.
Over the years it became crystal clear that both the general public and politicians have questions about the use of genetically modified plants in agriculture. People have questions about their food safety, and whether there is a real risk that genetically modified potatoes will spread in nature or might exchange genes with other varieties in the field. Will it be possible for organic farming to co-exist with the cultivation of genetically modified potatoes? Will there be an effect on bees and what about the protection of intellectual property?

A new product, different concerns

Food safety of genetically modified Phytophthora resistant potatoes

It is crucial to consider the technology and the applications separately in the discussion about food safety. Regarding the genetic modification technique there is a scientific consensus that there is nothing inherently unsafe in the technology. Thirty years of research and hundreds of studies demonstrate this, including long term studies and multigenerational feeding trials.\textsuperscript{48-51} Dozens of scientific academies and food safety agencies over the world further substantiate this consensus, as do organisations as the World Health Organisation.\textsuperscript{52} It is fair to say that the consensus on the safety of GM technology is as overwhelming as the consensus on global warming. Does this imply that GM crops should not be tested at all prior to commercialisation? Absolutely not. The safety of the crops should be evaluated according to the application.

The only difference between the cisgenic Phytophthora resistant Bintje, Desiree or Maris Pipers and the conventionally bred varieties will be the presence of resistance genes originating in wild relatives of the modern potato. The late blight resistance genes are part of a huge family of genes with similar characteristics. Many plants we eat on a daily basis possess hundreds of copies of this type of gene.\textsuperscript{53} Our ancestors have eaten these genes and their gene products for ten thousands of years. There is thus a very long history of safe consumption of these resistance genes. Moreover, the resistance genes that will be introduced in Bintje, Desiree and Maris Piper are identical and/or very similar to the resistance genes present in the traditionally bred varieties Toluca, Bionica and Sarpo Mira. For several years these varieties have been used in organic farming, with a history of safe use. Additionally, the resistance genes produce such extremely low amounts of protein that it is almost impossible to detect them. With this evidence that genetic modification is a safe technology, and given that in this project only potato genes with a long history of safe use will be transferred, there is no valid argument to consider that genetically modified resistant varieties are less safe than the existing traditionally bred late blight resistant varieties.

This does not mean that the genetically modified potatoes developed in this project will not be tested. The concentration of certain unhealthy compounds (as glycoalkaloids) naturally present in all potatoes will be measured to make sure that the concentration doesn’t exceed preset thresholds. In Europe genetically modified crops must successfully pass many additional tests. The plants should not only be molecularly characterised in detail but also the composition, toxicity and allergenicity need to be analysed.

Genetically modified Phytophthora resistant potatoes and the environment

GM Phytophthora resistant potatoes hold genes that are naturally present in wild potato species. As mentioned before huge amounts of these and comparable genes are present in conventionally bred Phytophthora resistant potatoes, and also in other crops.\textsuperscript{54} These genes will be equally environmentally safe in the genetically modified potato as the same genes are in conventionally bred varieties and in wild relatives. From the
cultivation experience of the Bionica, Toluca and Sarpo Mira varieties it is known that the late blight resistance genes do not cause an extra pressure on the environment and that the plants holding these genes are not invasive. The only change as a result of the introduction of the late blight resistance genes will be the interaction between the plant and the fungus like organism. The only difference the genes will make is that Phytophthora will no longer be able to infect and proliferate among the resistant potatoes; this is of course the desired change.

Another frequently mentioned concern is that the added genes could be transmitted to plants outside the field which would lead to the establishment of the trait in the environment. However, spreading of genes is independent of the technology by which the genes were introduced to plants. In other words the probability that genes will be distributed in the environment is the same for a classically bred variety as for a genetically modified one.

More importantly there are no wild potatoes in Europe. This means that transmission of a trait to a wild potato is therefore impossible in Europe. Nor does the potato have other sufficiently close evolutionary relatives in Europe with which it can cross. Black nightshade (Solanum nigrum) is the closest relative of the potato in Europe, and is unable to cross with potato.53 The potato can only cross with other cultivated potato varieties. However, even this chance is very small because cross breeding among commercial potato varieties is relatively rare.61 Depending on the variety potatoes are self-fertilising for 80% to 100% and thus do hardly accept pollen from other varieties. Additionally many varieties - for instance Binre - are as good as sterile.62 Even if the genes are transmitted by a cross to another potato variety then the late blight resistance genes would only be present in the DNA of the potato seed, and not in the harvestable tubers. A field of non-genetically modified potatoes would then still deliver a traditional non-GM produce. Even if the late blight resistance trait would only be able to spread if there were viable seeds that germinate and develop into plants that produce tubers in a potato field of the following year. However this chance is also very small.63 Most of the cultivated varieties in Europe are not able to produce viable seeds,64 and if they do then the germinated plantlets are extremely fragile.

To summarise:

- the GM late blight resistant potatoes will possess genes from wild potatoes of which some genes are already present in classically bred varieties;
- establishment of the GM trait in the environment is very unlikely since the GM late blight resistant potatoes have no wild relatives in Europe with which they can cross;
- interference with non-GM potato fields is very unlikely since:
  - the used potato varieties produce almost no pollen;
  - pollenisation with GM pollen will only change the genotype of the seeds and not the harvested potatoes;
  - cultivated varieties hardly produce viable seeds;
  - volunteer potatoes from seeds or non-harvested tubers are removed because of the strict crop rotation scheme specific to potato cultivation;

Moreover because of the nematode problems that potatoes face, potatoes will not be cultivated two consecutive years on the same field. For potatoes there is a strict crop rotation to ensure that potatoes are grown maximally once in three years on the same plot. If germ plants would be available in year 1 they will be treated as weed in year 2 and therefore eliminated mechanically or chemically.

WHAT ABOUT BEES AND HONEY?

Given that the late blight resistance trait is the only difference between genetically modified late blight resistant varieties and their equivalent non-GM varieties and given that the late blight resistance genes are not harmful for bees, there is no reason to anticipate an effect of the cultivation of the GM potatoes on bee health. Moreover bees do not visit potatoes because the flowers do not produce nectar. Potato pollen – on the condition that the flowers are male fertile – is spread by the wind or by insects as bumble bees and beetles but not by bees. Consequently bees will not come in contact with pollen of genetically modified potatoes.65 This statement was confirmed in Flemish fields by the Institute of Agricultural and Fishery Research (ILVO). During the potato field trial in Wetteren (Belgium) neighbouring bee keepers were invited to analyse honey samples for the presence of pollen of genetically modified potatoes. Not a single sample had traces of potato pollen, genetically modified or otherwise.66,67

Bees are our most important pollinators in agriculture and horticulture. In recent years their global health has suffered heavily. For example during the winter 2012-2013 one in three Belgian bee hives died.68 But the genetically modified crops that are commercially grown today cannot be linked in any way with the increased bee deaths. The decline of the global bee population is probably due to a combination of several detrimental circumstances, including the varroa mite, the use of pesticides in agriculture, and a shortage of energy rich food.69
What about intellectual property rights?

We face huge agricultural challenges, with increasing global population and standard of living, with climate change, and with the increasingly significant impact of agriculture on the environment. Investing in innovation is of major importance to provide solutions. However long-term investments can only be attractive if there is a financial return. The development of a new potato variety through traditional breeding techniques takes at least ten years and costs at least €3 million per variety. The variety creates added value for quality, yield and/or consumers’ health. In return, plant breeders want to be compensated to recover the financial investments. Plant breeders’ rights were created to address this.

Plant breeders’ rights - also known as plant variety rights - are rights granted to the breeder of a new variety. They give the breeder exclusive control over the propagation of that variety and the harvested material for a set number of years. With these rights the breeder can prevent somebody else from propagating the new variety or bringing it onto the market under a different name without the breeder’s approval. These rights enable the breeder to impose certain conditions, for example financial recompensation: for every plantlet that is propagated the breeder receives a defined compensation or royalty. This financial guarantee stimulates breeders to innovate. Applying for breeders’ rights is current practice in the potato breeding industry, providing protection for 30 years. This is also the case for the traditionally bred late blight resistant varieties Toluca, Bionica and Sarpo Mira.

Traits that are introduced into genetically modified crops are often protected with a patent instead. Similar to plant breeders’ rights a patent is a kind of intellectual property protection but it provides a stronger control compared to plant breeders’ rights (see Text box ‘Patents versus plant breeders’ rights’). Most of the late blight resistance genes used to develop the genetically modified potatoes are protected through patents by the parties that characterised the genes. Genetically modified potatoes containing patented genes can only be commercialised with the permission of the patent holder(s).

The Belgian potato consortium for example has to take a commercial license in order to be allowed to develop the GM late blight resistant potato variety Bintje PLUS for the market. The late blight resistance genes used to develop Bintje PLUS stay protected by the original patent holders as long as the patents are valid, however the Belgian researchers won’t protect Bintje PLUS with an additional patent. As for any new potato variety, plant breeders’ rights will be applied for. Consequently, once the original patents on the resistance genes are no longer valid other breeders will have the possibility to use Bintje PLUS as plant material in their breeding programmes. If Bintje PLUS itself would also be protected by a patent, permission of the consortium would be needed and the protection would last longer.

Currently, discussions are ongoing in the EU to implement a limited breeders’ exemption in the European patent right. This would mean that a permission of the patent holder is only needed at the moment a new variety (that was developed using a patented variety) is ready for the market. Now this permission is already needed during the development phase. This hurdle could potentially slow down innovation, which is conflicting with the idea behind intellectual property rights. The Netherlands and Germany already implemented this limited breeders’ exemption rule.
A late blight resistant potato for Europe

The underlying principle of a patent - that a breeder who develops a new potato variety with improved characteristics should get compensated - is identical to that of plant breeders’ rights (see Table 1, page 35). The implementation and specifications differ in that a patent provides stronger control than plant breeders’ rights. Breeders’ rights can be used to protect a variety, in other words the complete package of characteristics. In contrast a patent protects one or more specific characteristics independently of the genetic background of a variety.

Additionally there is a significant difference concerning the protection and use of farm-saved seeds and/or propagated plant material as tubers. Within plant breeders’ rights the use of harvested material (seeds or other material) is allowed for twenty-two crops, including potato.61 If a farmer decides to keep a part of its own potato harvest to use as planting material for the next season, they should announce that to the holder of the plant variety certificate. In return for using farm saved plant material the farmer has to pay a ‘fair’ compensation. The latter is a percentage of the original licence fee or royalty. The fee is set by the breeder and depends on the crop and variety. For potato varieties this fee is usually half of the original royalty.62 For example, a Flemish farmer who uses farm saved potatoes (that are protected by plant variety rights) as planting material has to pay an average 100 euro per hectare.63 Only small-scale farms (less than four hectare of potatoes) do not have to pay compensation.64 In contrast, with patent rights there are no exceptions. For every type of commercial use a permission of the patent holder is needed. In practice patent holders usually do not approve part of the harvest being used as farm-saved seeds or planting material for the next season. As a result the farmer has to buy fresh material every year. An additional difference between plant breeders’ rights and patents concerns the use of protected varieties and traits by fellow breeders. Plant breeders’ rights tolerate breeders using each other’s varieties in their breeding programmes without each other’s permission or financial compensation being needed. For plant traits which are protected by patents this is not possible. In all cases a breeder needs the permission of the holder of the patent as long as the patent is valid.

<table>
<thead>
<tr>
<th>PATENTS VERSUS PLANT BREEDERS’ RIGHTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>The invention can only be used commercially with the permission of the patent holder</td>
</tr>
<tr>
<td>Granted for a period of 25 to 30 years</td>
</tr>
<tr>
<td>Only in those countries that granted the plant variety rights</td>
</tr>
<tr>
<td>Farm-saved seeds are forbidden except for 22 crops, if used a fair compensation should be paid</td>
</tr>
<tr>
<td>Variety is protected</td>
</tr>
<tr>
<td>Use in breeding is allowed</td>
</tr>
</tbody>
</table>

Table 1. Similarities and differences between plant breeders’ rights and patents

Co-existence or how can different cultivation practices exist next to each other?

Co-existence implies the parallel but separate use of distinct methods, for example organic and conventional farming. Both the consumer and farmer have the right to make a deliberate choice between these cultivation and production methods, so it should be ensured that the probability of mixing products will be reduced to a minimum. This is important since organic farming forbids use of genetically modified crops.

It is fairly simple to prevent a significant level of mixing, especially for the late blight resistant Bintje. As already described on page 30,31 Bintje is almost 100% sterile. In other words the potato produces only a limited amount of pollen or even no pollen at all. Consequently the probability that these potatoes will spread their genes to a neighbouring field is very small. Even if there would be cross breeding, the harvest will not be affected since the genes will only be incorporated in the potato berries and not in the tubers. Fertilised berries or GM potatoes that remained on the field could germinate and grow the following year, producing volunteer plants. However these plants will emerge in another crop because potatoes are never grown in two consecutive years on the same plot. As a result the germinated and/or volunteer potatoes will be taken for weeds and subsequently removed chemically or mechanically. Based on the biology and the cultivation method of potatoes a significant mixing is thus very unlikely.

However harvest and post-harvest procedures have to be followed to prevent mixing as a result of human negligence. To guarantee separate distribution channels, strict rules and engagements are needed regarding cleaning of harvesting machines between two fields and responsible handling of potato lots post-harvest.

A late blight resistant potato for Europe
Conclusion

Phytophthora infestans significantly affects European potato production. The potato disease caused by this fungus-like organism causes huge economic losses each year and current conventional prevention methods have a negative impact on the environment. Strict spraying schemes that farmers have to follow means that cultivation of potatoes is a stressful and labour intensive occupation.

European scientists are breeding potato varieties for resistance against the potato disease. They use both traditional breeding techniques and genetic modification. Cultivating Phytophthora resistant potatoes is the ideal and only sustainable way to circumvent this disease.

Similar to the scientific consensus on climate change, there is an overwhelming consensus on the fact that genetic modification is as safe as any traditional breeding technique. Given the urgent need for an efficient, sustainable solution to potato late blight, we cannot afford to ban a safe and effective technology. The method of breeding should be considered secondary to the overall goal of developing late blight resistant potatoes.
References


20. ILVO (persoonlijke communicatie van ongepubliceerde data.


Basic research in life sciences is VIB’s raison d’être. On the one hand, we are pushing the boundaries of what we know about molecular mechanisms and how they rule living organisms such as human beings, animals, plants and microorganisms. On the other, we are creating tangible results for the benefit of society. Based on close partnership with four Flemish universities – Ghent University, KU Leuven, University of Antwerp and Vrije Universiteit Brussel – and supported by a solid funding program, VIB unites the expertise of 74 research groups in a single institute. VIB’s technology transfer activities translate research results into new economic ventures which, in time, lead to new products that can be used in medicine, agriculture and other applications. VIB also engages actively in the public debate on biotechnology by developing and disseminating a wide range of science-based information about all aspects of biotechnology.

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The Sainsbury Laboratory (TSL) is a world-leading research centre focusing on making fundamental discoveries about plants and how they interact with microbes. TSL not only provides fundamental biological insights into plant-pathogen interactions, but is also delivering novel, genomics-based, solutions which will significantly reduce losses from major diseases of food crops, especially in developing countries. TSL is an independent charitable company and receives strategic funding from the Gatsby Charitable Foundation with the balance coming from competitive grants and contracts from a range of public and private bodies, including the European Union (EU), Biotechnology and Biological Sciences Research Council (BBSRC) and commercial and charitable organisations www.tsl.ac.uk